EXHIBIT 3
(PART 2 OF 2)

[0074] A functional assay would enhance classification into benign and deleterious mutations and aid in clinical interpretation. Gene expression profiling can be used as a functional assay to help define the clinical significance of variants. The present invention provides methods for analyzing and classifying mutations in genes that are individually linked to BRCA1 function and collectively provide a profile of such function from formalin-fixed, paraffin-embedded archival tissue samples.

Targeted therapies; personalized medicine

[0075] There is an opportunity for targeted treatment of germline-mutated BRCA1 breast cancers and the 15-30% of sporadic breast cancer with somatic inactivation of BRCA1 by mechanisms including DNA methylation or other epigenetic events. Breast tumors with loss of function of BRCA1 or BRCA2 are uniquely sensitive to PARP [Poly(ADP-ribose) polymerase] inhibitors due to underlying defect in DNA double-stranded break repair.

Need to identify patients as having BRCA1 loss of function in a timely manner

[0076] In order to provide targeted treatments, it will be crucial to identify patients with breast tumors that have BRCA1 loss of function at the time of diagnosis in order to provide targeted treatment. Comprehensive BRCA1/2 testing has a routine turn-around time of 3-4 weeks which may be too long for a timely diagnosis of inherited loss of BRCA1 function. While a rapid turn around time (7-10 business days) test is available the cost is an additional $1500, which is not borne by insurance. Gene testing will not identify somatic (acquired) loss of BRCA1 function. Therefore a rapid, pre-screen will be highly useful for identifying breast tumors with BRCA1 loss of function in a timely manner in order to use this information to guide targeted treatments.

Chemoprevention

[0077] Prevention of breast cancer in women at high risk can be accomplished by identifying women with breast cancer risk factors and instituting treatment. Several chemoprevention trials have been conducted which have demonstrated the preventive efficacy of selective estrogen receptor modulators (SERMs) such as tamoxifen and raloxifene. These agents have been FDA

approved for breast cancer chemoprevention in high-risk women. While SERM treatment results in a ~50% reduction in breast cancer risk, these agents are not tailored to the underlying risk factors in individual women. Agents with higher efficacy and lower toxicity are desirable. Furthermore, there is uncertainly as to whether SERMs are effective in BRCA1 mutation carriers, because SERMS are effective in the prevention of estrogen receptor positive breast cancers, but the majority (80-90%) of BRCA1 associated breast cancers are estrogen receptor negative.

[0078] BRCA1 mutation carriers have one wild-type allele and one mutated allele in each cell. If the wild-type allele is lost (e.g. through mutation or epigenetic modification, perhaps due to carcinogenic exposures) then the cell in which this occurs acquires a defect in DNA DS break repair. Subsequent mutational events lead to a clinically detectable cancer. The biological progression from a single cell with two hits in BRCA1 (germline and somatically acquired hits in each allele) to clinically detectable cancer represents an ideal time in which to institute chemopreventive treatments. PARP inhibitors may provide an ideal chemopreventive treatment for BRCA1 mutation carriers because the agents are specifically targeted to the underlying defect, have a high therapeutic index (high efficacy against disease coupled with low toxicity to non-cancerous cells), and can eliminate the very first cell that arises in the cancer progression pathway.

[0079] PARP inhibitors are now in use in clinical trials in combination with cytotoxic drugs (Jagtap P, Szabo C. Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors. Nat Rev Drug Discov 2005; 4(5):421-440). Clinical trials using PARP inhibitors for *BRCA* carriers have been discussed in the literature (Tutt AN, Lord CJ, McCabe N et al. Exploiting the DNA repair defect in BRCA mutant cells in the design of new therapeutic strategies for cancer. *Cold Spring Harb Symp Quant Biol* 2005; 70:139-148). The 2007 American Society of Clinical Oncology meeting (6/07, Chicago, IL) included a report of phase I study of PARP inhibitors.

[0080] Gene expression profiling would be useful in at least two chemoprevention scenarios for BRCA1 mutation carriers. First, women who have had already had breast cancer because of an underlying BRCA1 mutation are at very high risk of another breast cancer, about 40% during the first ten years following an initial diagnosis of breast cancer. Performing gene expression

profiling on their tumors, and confirming the presence of an inherited BRCA1 mutation through gene testing, would make possible the use of a selective chemoprevention agent such as a PARP inhibitor. Chemoprevention agents that are not known to be targeted to BRCA1 breast cancers, such as SERMs, may also be utilized for treatment. Because BRCA1 mutation carriers have a high incidence of bilateral breast cancer, chemoprevention of a second breast malignancy would be an important addition to the armamentarium of treatments.

[0081] Second, by identifying probands with BRCA1 mutations using gene expression profiling, unaffected relatives can be identified and chemoprevention instituted in them prior to the development of a primary breast cancer. Chemoprevention may include agents that are targeted to BRCA1 mutations carriers, such as PARP inhibitors, or agents such as SERMs.

Breast cancer stem cell biology

[0082] Gene expression profiling of tumors with BRCA1 loss of function can be used to shed light on breast cancer stem cell biology. Foulkes and others have advanced a hypothesis that BRCA1 functions as a breast stem cell regulator. The cancer stem cell theory posits that a self-replenishing pool of stem cells gives rise to cancer and that cancer treatments may leave part of this pool untouched, serving as a source for cancer recurrence. Identifying BRCA1 breast tumors using gene expression profiling and studying these tumors may be useful in order to develop experimental models of stem cell regulation and improved therapies. The specific genes which define the BRCA1 profile are over-represented by genes involved in stem cell regulation in a variety of tissues. These may serve as targets for early detection assays of breast cancer as well as therapeutic targets. See Foulkes WD, *BRCA1* functions as a breast stem cell regulator. *J Med Genet*, 2004, 41:1-5.

EXAMPLES

Example 1 Samples and RNA preparation

[0083] Formalin-fixed, paraffin embedded (FFPE) tissue blocks were retrieved from the pathology archives bank of Evanston Northwestern Healthcare (Evanston, IL), Department of Pathology in accordance with HIPAA and Institutional Review Board (IRB) guidelines. Total RNA was prepared from the FFPE breast tumor blocks using the High Pure RNA Paraffin Kit

(Roche Applied Science, Indianapolis, IN). All chosen blocks contain more than 50% tumor. The relationship of RNA quality versus the age of the archival sample is illustrated in **FIG. 4**. As the data indicates, the age of the archival material is not predictive of sample quality. The oldest sample in the study (39 years) demonstrates one of the highest quality RNAs

Example 2 Microarray analysis and DASL™ RNA pre-qualification

[0084] RNA extractions were pre-qualified for the DASL™ assay by a real-time PCR assay recommended by Illumina Inc. (Illumina: Gene Expression on Sentrix Arrays: DASL Assay System Manual, *Doc # 11175105 edn: Illumina Inc* 2004). RNA (200 ng) was reverse-transcribed into cDNA using the Master Mix for cDNA synthesis, single use reagent (Illumina, San Diego, CA). The rtPCR reactions were performed on an ABI Prism 7900HT Real Time System (Applied Biosystems, Foster City,CA) using a Platinum® SYBR® Green qPCR superMix-UDG with Rox (Invitrogen, Carlsbad, CA) with the recommended PCR program and primers [1] to yield a 90 bp transcript-specific fragment of the highly expressed RPL13a ribosomal protein gene (GenBank accession # NM_012423.2).

Example 3 DASL™ gene expression

[0085] In the DASL™ assay total RNA is converted into cDNA using a reverse transcription reaction using random hexamers and is then labeled with biotinylated oligos ($b(N)_9$ and $b(T)_{18}$). Pairs of query oligonucleotides are annealed to complementary sequences (~50 bases) flanking specified cDNA target sites. The biotinylated cDNA is then bound to streptadadivin particles and washed to eliminate mis and non-hybridized particles. A primer extension and ligation process then forms a biotinylated (~100 bp) DASL product containing a unique address sequence for a specific gene. This product is then amplified using conditions detailed in [1] and two of three universal primers to produce a fluorescently labeled amplicon for hybridization. The two upstream primers are 5' labeled with Cy3 and Cy 5 respectively while a downstream primer is biotinylated for capture and elution of the PCR product. The use of two dyes results in two separate measurements of a transcripts population and thus increases statistical power.

[0086] Labeled amplicons are hybridized to a BeadChip or a Sentrix Array Matrix in an oven overnight while cooling from 60 to 45 degrees Celsius. The arrays consist of etched pits

populated by silica beads with complimentary unique address codes. Each array contains about 50,000 3 μm silica bead which results in each unique address or bead type (1536) being present about 30 times per array. The beads are positioned randomly, and a decoding procedure is used to identify the location and DNA sequence on each bead (Oliphant A, Barker DL, Stuelpnagel JR, Chee MS: BeadArray technology: enabling an accurate, cost-effective approach to high-throughput genotyping *Biotechniques* 2002, (Suppl):56-58). After hybridization, the array is then scanned by laser confocal microscopy using an automated BeadStation™ Reader and SentrixScan™ software from Illumina. The software creates an intensity data file which is used in statistical analysis of the results.

Example 4 Procedures for BRCA1 promoter methylation

[0087] DNA methylation analysis of the BRCA1 promoter was performed to investigate the basis for reduced expression in the absence of gene mutation. One 5μm tissue section was cut from each FFPE block and DNA was isolated using the PUREGENE DNA Purification Kit (Gentra System, Minneapolis, MN). PCR amplification of a 223 bp human DNA target was performed to assess DNA quality, which was good in all cases. DNA samples were then bisulfite treated using EZ DNA Methylation-Gold kit (Zymo Research Corp., Orange, CA). We used CpGenome Universal Methylated DNA (CHEMICON International Inc., Temecula, CA) as positive control and a normal sample as negative control. BRCA1 methylation status was determined by methylation-specific PCR. Primer sequences (3272bp-3360bp) were 5'-gAgAggTTgTTgTTTAgCggTAgTT (forward) and 5'-CgCgCAATCgCAATTTTAAT (reverse) and probe oligo sequence was 5'-6FAM-CCgCgCTTTTCCgTTACCACgA—TMR (Widschwendter M, Cancer Res 2004; 64: 3807-3813). Methylation-specific PCR was carried out in 20ul reaction volumes on a Roche Lightcycler (Roche Applied Science) for 50 cycles (10s at 95°C, 30s at 64°C, 20s at 72°C).

Example 5 Quantitative RT-PCR

[0088] To further confirm our microarray data, we performed qRT-PCR on three genes showing differential expression. Total RNA was prepared from the FFPE breast tumor blocks using the High Pure RNA Paraffin Kit (Roche Applied Science) and converted to cDNA using RT² PCR Array First Strand Kit (SuperArray Bioscience Corporation, Frederick, MD). A total of

500ng tRNA for each sample was used to prepare cDNA according to the manufacturer's instructions. Human Universal Total RNA (SuperArray Bioscience Corporation) was used as a positive control, and for construction of standard curves to quantify each gene. The primers for MAGEA4, SPIB, BRCA2, and GAPDH (used as a housekeeping gene for normalization) were obtained from SuperArray Bioscience Corporation.

[0089] RT-PCR was performed in 20ul reaction volumes on a Roche Lightcycler (Roche Applied Science) with amplification for 50 cycles (30s at 95°C, 30s at 55°C, 30s at 72°C). Each reaction was subjected to melting point analysis to confirm single amplified products.

Example 6 Adaptation of gene expression profiling of BRCA1 breast tumors to archival materials

[0090] Fresh frozen tissue is the specimen type used in all prior art in BRCA1 gene expression profiling. Limited numbers of fresh frozen tissue specimens are available for *BRCA1* research studies and virtually none are available for clinical use. This is because pathology laboratories prepare and archive tumors in the form of formalin-fixed, paraffin-embedded (FFPE) tissues. A vast number of such specimens exist in clinical pathology laboratories across the United States and around the world, which are usually stored for a decade or longer.

[0091] The Illumina DASL (cDNA-mediated annealing, selection, extension and ligation) assay is designed to generate reproducible profiles from degraded RNAs, for example FFPE archival specimens. We selected a select limited number of target genes for custom array. Each gene represented by 3 oligonucleotide probes. Each probe is represented by approximately 30 beads.

[0092] Sample prequalification was done using RT-PCR of a housekeeping gene. Most samples were 1-2 decades old; some were 3-4 decades old.

The design of the 128-gene DASL array

[0093] As a first step in designing the array, we performed an extensive literature search on BRCA1-related breast tumor studies. Many of such studies involve DNA microarrays and various gene lists that related to BRCA1. The compiled list includes 354 unique genes.

[0094] We further selected 721 genes that are differentially expressed BRCA1-mutated tumors according to our own DNA microarray data. A pooled RNA sample representing BRCA1-mutated breast tumors and another pooled sample representing sporadic tumors were hybridized three times to Affymetrix U133 Plus 2.0 array. We observed that 21 genes are common to this list and the list from literature review. Two genes/ESTs (BQ707388 and AL137761) could not be mapped to RefSeq using http://david.abcc.ncifcrf.gov/ and were not used in the final array. Thus, **19** of these **21** genes are included in our array.

[0095] We retrieved the expression data on literature-based gene list from the microarray data of van t' Veer et al (Nature 2000) that covers BRCA1-mutated (20 samples) and sporadic breast tumors (96 samples). Similar data is also retrieved from our own DNA microarray data based on pooled samples of mutated and sporadic breast tumors. We then ranked these genes by their correlation with the BRCA1 vs. sporadic distinction. A correlation coefficient is calculated by, where μ_1 and σ_1 are the mean and standard deviation of the expression level in BRCA1 mutated samples, μ_2 and σ_2 are corresponding parameters for sporadic samples (Golub et al., Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science* 1999 Oct 15, 286:531-7). Genes are ranked according to this parameter. We selected based on the average of the percentile from both datasets. We selected **31** genes that are highly expressed in BRCA1-mutated tumors and **30** genes highly expressed in sporadic tumors. Of the 31 genes that are highly expressed in BRCA1 mutated tumors, one (AK126320) could not be mapped to RefSeq using http://david.abcc.ncifcrf.gov/ and was not used in the final array. Of the 30 genes that are highly expressed in sporadic tumors, one (AK096661) could not be mapped to RefSeq using http://david.abcc.ncifcrf.gov/ and was not used in the final array.

[0096] A total of four gene substitutions were made based on inability to map genes/ESTs (BQ707388, AL137761, AK126320, AK096661) to RefSeq. The following genes were substituted in lieu of these gene/ESTs: COX6C and PDGFB (both highly expressed in BRCA1-mutated tumors) and TOPBP1 and MCM7 (both highly expressed in sporadic tumors).

[0097] Two of the 30 genes that are highly expressed in sporadic tumors had a low predicted probability of success on the array according to the probe design analysis performed by Illumina,

Inc. These two genes, PRSS2 and DKF2p434E2321 were not used in the final array. Two gene substitutions were used, PDGFRB and CD36, both of which were also highly expressed in sporadic tumors.

[0098] To overcome the limit of the literature reported genes, we also included the top **10** genes that are highly expressed in BRCA1 mutated tumors and top **10** genes lowly expressed in such tumors (which corresponds to "high in sporadic") according to our own expression data. For the "top **10** genes that are highly expressed in BRCA1 mutated tumors", one (FABP7) was also included in the "overlapping 21 genes" category leaving **9** independent genes in this category.

[0099] Another **20** genes are selected based on their biological relevance with BRCA1-mutated breast tumors. Such genes include the genes like BRCA1 and BRCA2, and also various keratin genes that are known to be important in distinguishing different types of breast tumors. Of these 20, **19** are noted independently of the other selection criteria; 1 (ESR1) overlaps with "overlapping 21 genes".

[00100] For quality control purposes, we included the following 5 housekeeping genes as positive controls: ACTB, GAPD, EIF4G2, SRRM1, and KHDRBS1. Two of them (ACTB and GAPD) are highly expressed, and the other three (EIF4G2, SRRM1 and KHDRBS1) are expressed at moderate levels. Furthermore, we included 3 genes as negative controls that are not expected to be expressed in breast tissues. Such genes include a brain-specific gene (MAG), a liver-specific gene (CFHL5), and a colon-specific gene (CEACAM1).

[00101] These selections gave a final total of 128 genes for the custom array, which are detailed in **TABLE 3** and **TABLE 4**. The distribution of genes selected for the custom array fall broadly into several functional categories, with particular weighting towards transcriptional regulation, cell cycle control, and DNA repair (**FIG. 5**).

Example 7 Confirmatory Studies

[00102] Quantitative real-time PCR was performed for three genes (MAGEA4, SPIB, BRCA2) to confirm gene expression found on microarray analyses. MAGEA4 (melanoma

antigen family A, 4) was selected for analysis based on its extremely high expression (greater than 10 fold) in 50% of the $BRCA1^+$, ER^- tumors (**FIG. 6**). MAGEA4 is a tumor antigen that is known to be related with other cancer types (e.g. germ cell tumors, malignant melanomas, certain carcinomas and sarcomas).

[00103] SPIB (Spi-B transcription factor) was selected for analysis based on its high fold change (2.5x) in BRCA1 mutated vs. sporadic breast cancers and its independence from estrogen receptor status (**FIG. 7**). SPIB is involved in the control of plasmacytoid dendritic cells development by limiting the capacity of progenitor cells to develop into other lymphoid lineages.

[00104] BRCA2 (breast cancer 2, early onset) was selected based on biological interest. Differential expression of BRCA2 was significant, but not high (1.6 fold, see **FIG. 8**). BRCA2 gene expression correlates with ER status (higher expression in ER^- breast tumors). BRCA2 germline mutations cause a very similar clinical syndrome compared to BRCA1, and genetic testing usually involves both genes. The biological characteristics of germline BRCA2-mutated breast cancers are distinct from germline BRCA1-mutated breast cancers. The scientific literature has not previously suggested coordinate regulation of these two genes.

[00105] MAGEA4 gene expression as measured by qPCR was highly correlated with MAGEA4 gene expression as measured by DASL array as indicated in **FIG. 9** (P value for sporadic vs. BRCA1: $P < 0.0054$ by Wilcoxon rank sum test with continuity correction).

[00106] As illustrated in FIG. 10, SPIB gene expression as measured by qPCR was highly correlated with SPIB gene expression as measured by DASL array (Student T-test P value <0.024. ER+ vs ER-: Wilcoxon test P <0.05).

[00107] BRCA2 expression, contrary to the confirmatory studies performed on MAGEA4 and SPIB, did not show a statistically significant correlation between the DASL expression results and the qPCR data (FIG. 11, sporadic vs BRCA1+: Wilcoxon test P <0.48, not significant. T-test also not significant). The low degree of differential expression between the sporadic and BRCA1+ samples and the lack of statistical correlation call into question whether the two BRCA2 probes are spuriously correlated with BRCA1 germline mutation status. For genes with

high-fold expression (MAGEA4 and SPIB), qPCR and DASL expression results are highly correlated, confirming the reliability of DASL gene expression results.

Example 8 Data Acquisition

A. Visualization

[00108] Raw image data is processed with Illumina's BeadStudio Version 1.5 to summarize gene expression in terms of a signal and a detection score. The detection score was calculated by comparing a signal produced by a probe with these produced by negative control probes, modeled by a normal distribution. A detection score >0.99, equivalent to P value <0.01, was used as a threshold for detected probes.

B. Quality Control

[00109] Microarray data was first subjected to a quality control process. Samples with less than 50% detected probes were removed from further analysis. These samples tend to have high background levels. We eliminated 10 out of 83 samples. The qualified 73 samples were divided into a training dataset of 43 samples and an independent testing dataset of 30 samples. The training dataset contains 21 BRCA1+ samples (7 ER+, 14 ER-) and 22 sporadic samples (8 ER+, 14 ER-). The same ER+ to ER- ratio is maintained in this training dataset in both BRCA1+ and sporadic groups to avoid possible biases. Sporadic samples that are hyper-methylated in the BRCA1 promoter were also excluded from training dataset.

C. Normalization

[00110] A background normalization method provided in BeadStudio software (Illumina: Gene Expression on Sentrix Arrays: DASL Assay System Manual. Doc No. 11175105 edn: Illumina, Inc. 2004) was used to subtract a constant background value from all expression values. To further reduce intra-chip variability, all-vs.-all LOESS normalization was performed using the "affy" package in Bioconductor (Oliphant A, Barker DL, Stuelpnagel JR, Chee MS. BeadArray technology: enabling an accurate, cost-effective approach to high-throughput genotyping. Biotechniques (Suppl.) 56-58, 2004). Parameters were normalize.loess (data, epsilon =1, log.it =F, span =0.4, maxit =2), where data is our data matrix.

D. Gene selection

[00111] Log-transformed data was used to perform a student t-test to select differentially expressed genes in BRCA1+ samples. Probes were selected if it has a P value < 0.01 and shows a minimal fold-change of 1.20. Probes more strongly associated with ER status, as indicated by P value, were excluded.

[00112] We selected 14 probes representing 13 genes (FIG. 2). Two probes of BRCA2 genes were included in this list, suggesting that this gene's association is robust. Gene Ontology analysis using DAVID (Database for Annotation, Visualization, and Integrated Discovery, see Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol. 2003;4(5):P3) revealed that this list is enriched (P<0.0003) with DNA repair related genes. Four genes in this list (MSH2, MSH6, TOPBP1 and BRCA2) are related to DNA repair.

E. Supervised classification of independent dataset

[00113] Based on the selected genes and the training dataset, an independent dataset was classified using k-nearest neighbor algorithm (KNN). Samples in both training and testing datasets are further normalized to have a mean of zero and standard deviation of 1. Weighted votes from six most similar samples are used to predict the class membership of a testing sample. If vote from two classes are close (difference less than 30%), no prediction will be made.

[00114] The algorithm correctly predicted the class membership (BRCA1+ or sporadic) of 25 (83.3%) out of 30 testing samples. Nine out of 11 BRCA1+ samples are consistently classified, equivalent to a sensitivity of 81.8%. Two out of 11 predicted BRCA1+ samples are false positives, leading to a specificity of 81.8%.

[00115] Leave-one-out cross validation was carried out by withholding one sample each time and select predictor genes to make predictions of the withheld sample. We observed an overall accuracy of 72%.

F. Reproducibility--- technical replicates

[00116] Technical replicates for 7 RNA samples were hybridized twice to test the reproducibility of the platform. We observed an average Pearson's correlation coefficient of R=0.851.

Example 9 Methylation Studies

[00117] DNA methylation of the BRCA1 promoter was observed in 10 of 28 (36%) sporadic breast cancers and 2 of 20 (10%) BRCA1 germline-mutated breast cancers. BRCA1 expression was analyzed with respect to DNA methylation status of the BRCA1 promoter for sporadic breast tumors (FIG. 3). BRCA1 expression levels were inversely correlated with methylation of the BRCA1 promoter ($P<0.01$).

[00118] Our results confirm previous reports that BRCA1 promoter methylation serves as a basis for reduced expression in the absence of gene mutation. We found a high level of BRCA1 methylation among sporadic breast tumors (36%) as compared with 15-30% reported in the literature. One implication of epigenetic BRCA1 modification would be that BRCA1-like sporadic breast cancers may be "misclassified" as BRCA1 germline-mutated, and could comprise a proportion of samples coded as false positive on the classifier. If classifier results were used to guide selection of patients for germline BRCA1 mutations analysis, this would be a source of negative germline sequence results.

[00119] Therefore we assessed whether false positive specimens, i.e. sporadic breast tumors that were misclassified as BRCA1 germline-mutated, were DNA methylated at the BRCA1 promoter. Neither of the two misclassified specimens demonstrated BRCA1 promoter methylation. We conclude that for our specimens, BRCA1 promoter methylation did not contribute to the imperfect specificity of the classifier. However, since BRCA1 promoter methylation has previously been shown to be associated with false positive misclassification in a gene expression profiling study (Hedenfalk et al. Gene-expression profiles in hereditary breast cancer. N Engl J Med 2001; 344(8):539-548), we hypothesize that BRCA1 promoter methylation can occur as an early or a late event in tumorigenesis, with different effects on tumor biology. We further hypothesize that if BRCA1 methylation occurs early during tumorigenesis, it may be

an etiologic factor that influences downstream events and leads to a BRCA1-like phenotype. In these sporadic breast tumors, the BRCA1 expression profile would parallel that of BRCA1 germline-mutated tumors. If the underlying biology of sporadic BRCA1-like and germline-mutated BRCA1 breast tumors are similar, then targeted therapies may be valuable for not only BRCA1 germline mutated breast cancers, but also the larger population of sporadic BRCA1-like breast cancers. This has important implications as targeted therapies (e.g. PARP inhibitors) are now in phase II trials for patients with germline BRCA1 mutated cancers. If these therapies are effective, they may also be applicable to the 15-30% of women with BRCA1-like breast cancers.

[00120] Our data also report for the first time on the methylation status of germline mutated BRCA1 breast tumors, showing BRCA1 promoter methylation of 10% of specimens. BRCA1 promoter methylation in germline-mutated tumors may serve as a second hit to silence BRCA1 express

Claims

1. A method for identifying *BRCA1* mutations in breast and ovarian cancer tissue comprising the use of gene expression profiles or patterns from archival formalin-fixed paraffin-embedded (FFPE) specimens using a DNA array.

2. The method of claim 1, wherein said gene profiling distinguishes between sporadic and hereditary types of breast and ovarian cancer.

3. The method of claim 1, wherein said method is independent of estrogen receptor (ER) status of the tissue.

4. The method of claim 1, wherein the gene profiling is selected from a group of 128 selected genes as reflected in TABLE 4.

5. The method of claim 1, wherein the DNA array is a DASL array.

6. The method of claim 1, wherein the sensitivity of detecting *BRCA1* mutations by using said gene profiling method is greater than 50%, and still further wherein the specificity for correctly classifying *BRCA1* mutations of said gene profiling method is equal to or greater than 60%.

7. The method of claim 1, wherein the sensitivity of detecting *BRCA1* mutations by using said gene profiling method is greater than 80%, and still further wherein the specificity for correctly classifying *BRCA1* mutations of said gene profiling method is equal to or greater than 70%.

8. The method of claim 1, wherein the sensitivity of detecting *BRCA1* mutations by using said gene profiling method is greater than 90%, and still further wherein the specificity for correctly classifying *BRCA1* mutations of said gene profiling method is equal to or greater than 80%.

9. A method for identifying *BRCA1* mutations in breast and ovarian cancer tissue comprising the use of gene expression profiles or patterns from archival formalin-fixed paraffin-embedded (FFPE) specimens using a DNA array, wherein said gene profiling distinguishes

between sporadic and hereditary types of cancer, and further wherein said method is independent of estrogen receptor (ER) status of the tissue, and further wherein the gene profiling is selected from a group of 128 selected genes as reflected in TABLE 4.

10. The method of claim 9, where at least 5 genes for the gene profiling are further selected from the 13 genes reflected in TABLE 5.

11. The method of claim 9, where at least 10 genes for the gene profiling are further selected from the 13 genes reflected in TABLE 5.

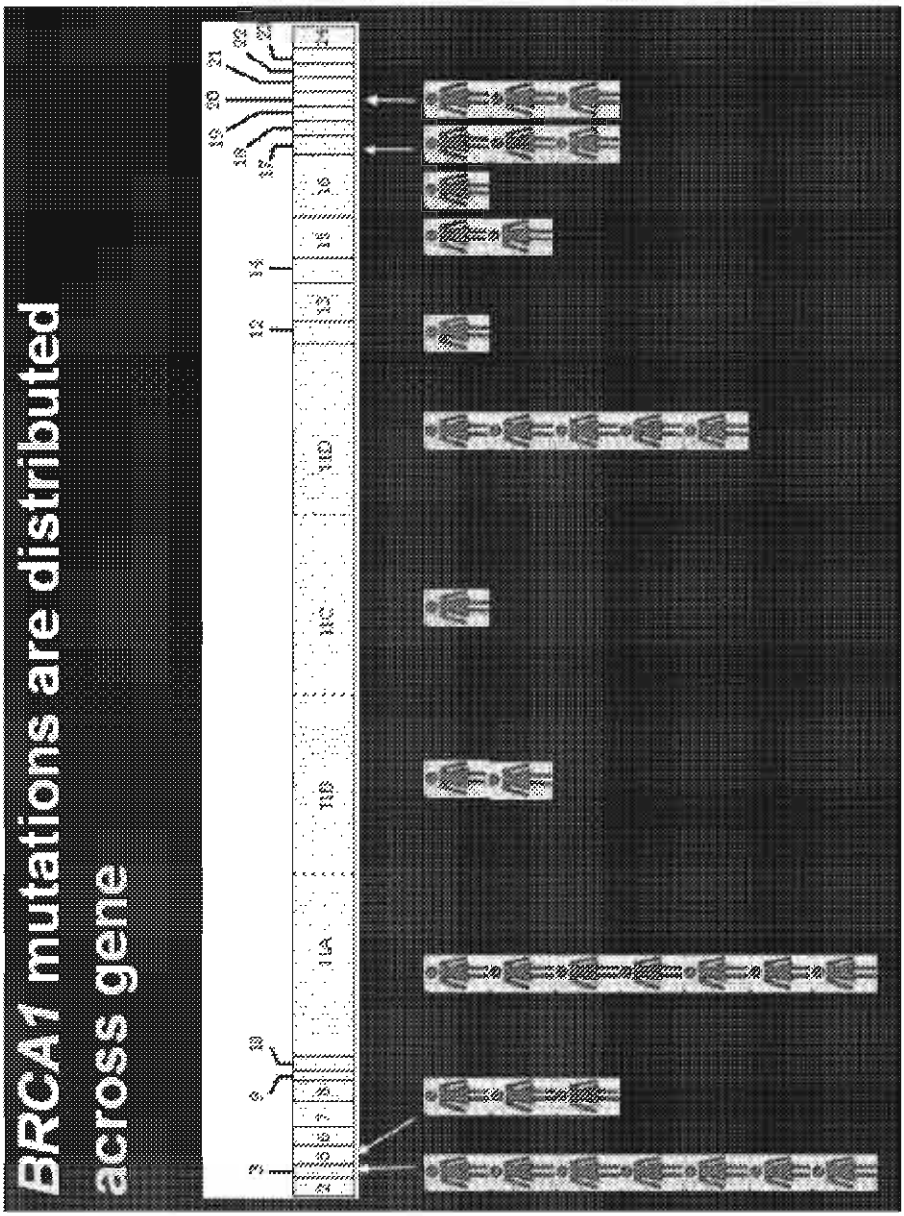
12. A method for identifying *BRCA1* mutations in breast and ovarian cancer tissue comprising the use of gene expression profiles or patterns from archival formalin-fixed paraffin-embedded (FFPE) specimens using a DNA array, wherein said gene profiling distinguishes between sporadic and hereditary types of cancer, and further wherein said method is independent of estrogen receptor (ER) status of the tissue, and further wherein the DNA array is a DASL array, and further wherein the gene profiling is selected from a group of 128 selected genes as reflected in TABLE 4, and further wherein the sensitivity of detecting *BRCA1* mutations by using said gene profiling method is greater than 70%, and still further wherein the specificity for correctly classifying sporadic *BRCA1* mutations of said gene profiling method is equal to or greater than 50%.

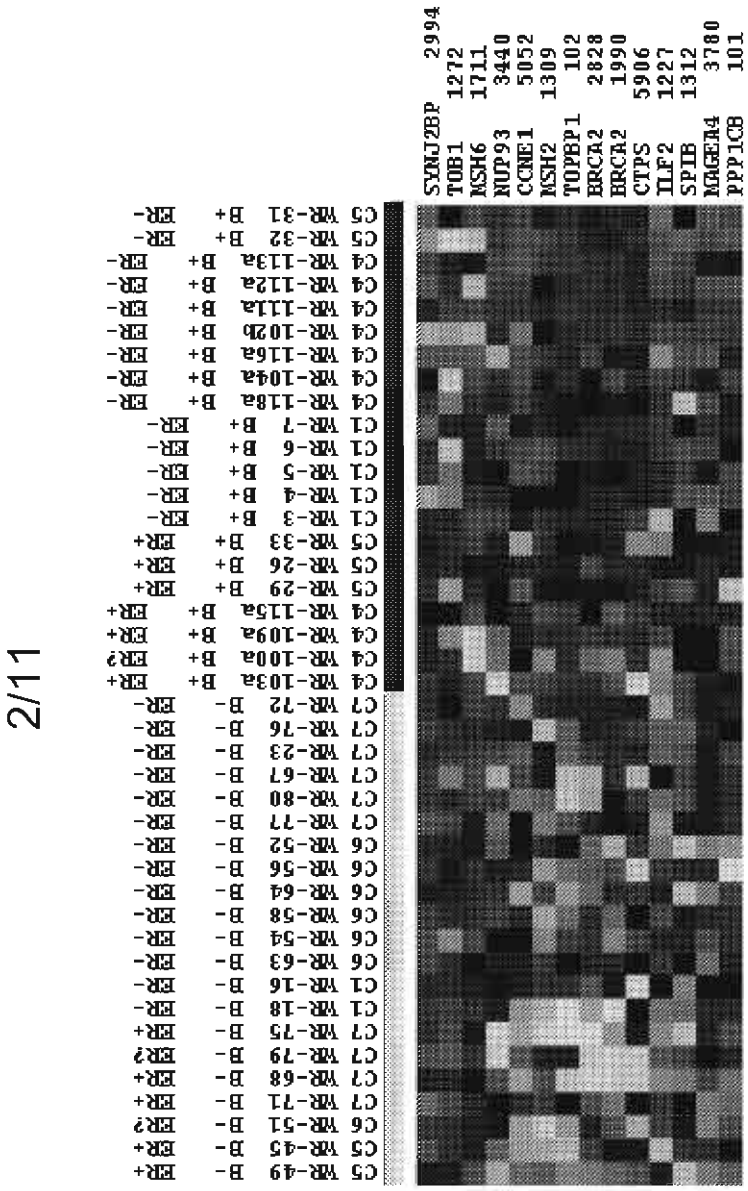
13. The method of claim 12, wherein the sensitivity of detecting *BRCA1* mutations by using said gene profiling method is greater than 80%, and still further wherein the specificity for correctly classifying *BRCA1* mutations of said gene profiling method is equal to or greater than 70%.

14. The method of claim 1, wherein the sensitivity of detecting *BRCA1* mutations by using said gene profiling method is greater than 90%, and still further wherein the specificity for correctly classifying *BRCA1* mutations of said gene profiling method is equal to or greater than 80%.

15. The method of claim 12, where at least 5 genes for the gene profiling are further selected from the 13 genes reflected in TABLE 5.

16. The method of claim 12, where at least 10 genes for the gene profiling are further selected from the 13 genes reflected in TABLE 5.


BRCA1 mutations are distributed across gene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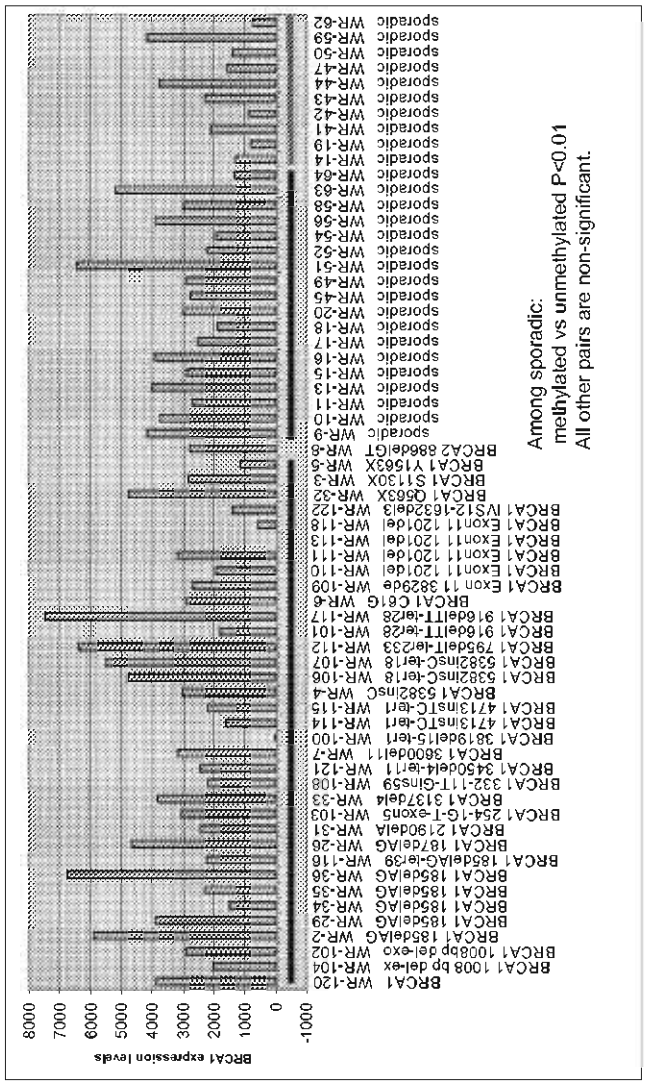
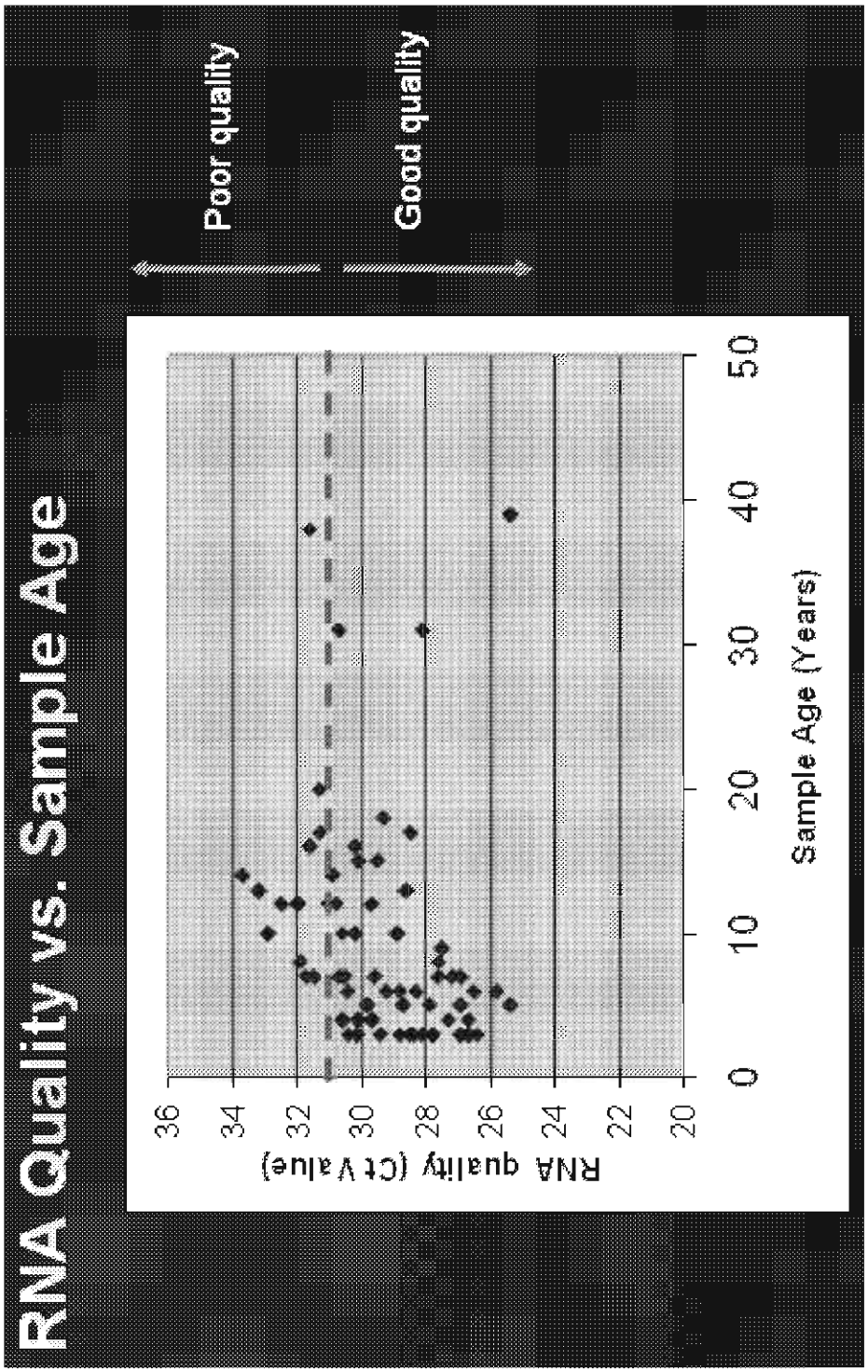
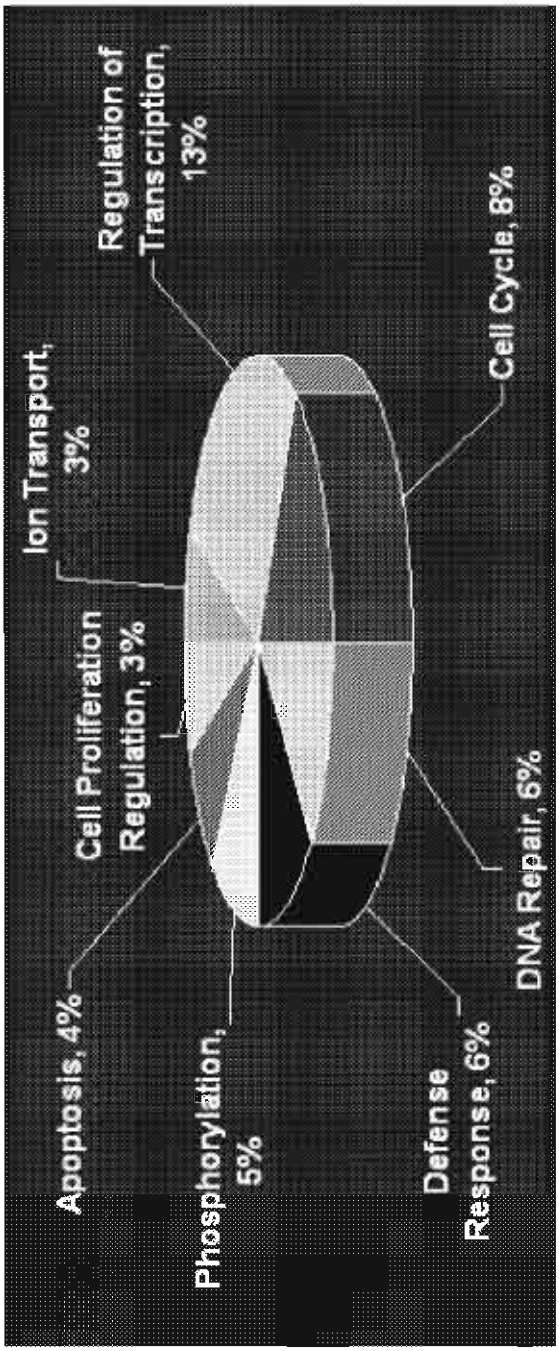

BRCA1+
sporadic
Non-methylated
Methylated
BRCA1 expression levels
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
BRCA1 WR-120
BRCA1 1008 bp del-ex WR-104
BRCA1 1008bp del-exo WR-102
BRCA1 185delAG WR-2
BRCA1 185delAG WR-29
BRCA1 185delAG WR-34
BRCA1 185delAG WR-35
BRCA1 185delAG WR-36
BRCA1 185delAG-ter39 WR-116
BRCA1 187delAG WR-26
BRCA1 2190delA WR-31
BRCA1 254-1G-T-exon5 WR-103
BRCA1 3137delA WR-33
BRCA1 332-1T-Gins59 WR-108
BRCA1 3450del4-ter11 WR-121
BRCA1 3600del11 WR-7
BRCA1 3819del15-ter1 WR-100
BRCA1 4713insTC-ter1 WR-114
BRCA1 4713insTC-ter1 WR-115
BRCA1 5382insC WR-4
BRCA1 5382insC-ter18 WR-106
BRCA1 5382insC-ter18 WR-107
BRCA1 7954delT-ter233 WR-112
BRCA1 9168delTT-ter28 WR-101
BRCA1 9168delTT-ter28 WR-117
BRCA1 C61G WR-6
BRCA1 Exon 11 3829de WR-109
BRCA1 Exon11 1201del WR-110
BRCA1 Exon11 1201del WR-111
BRCA1 Exon11 1201del WR-113
BRCA1 Exon11 1201del WR-118
BRCA1 IVS12-1632del3 WR-122
BRCA1 Q563X WR-32
BRCA1 S1130X WR-3
BRCA1 Y1563X WR-5
BRCA2 8864delGT WR-8
sporadic WR-9
sporadic WR-10
sporadic WR-11
sporadic WR-13
sporadic WR-15
sporadic WR-16
sporadic WR-17
sporadic WR-18
sporadic WR-20
sporadic WR-45
sporadic WR-49
sporadic WR-51
sporadic WR-52
sporadic WR-54
sporadic WR-56
sporadic WR-58
sporadic WR-63
sporadic WR-64
sporadic WR-14
sporadic WR-19
sporadic WR-41
sporadic WR-42
sporadic WR-43
sporadic WR-44
sporadic WR-47
sporadic WR-50
sporadic WR-59
sporadic WR-62
Among sporadic:
methylated vs unmethylated P<0.01
All other pairs are non-significant.


RNA Quality vs. Sample Age
Poor quality
Good quality
RNA quality (Ct Value)
36
34
32
30
28
26
24
22
20
0
10
20
30
40
50
Sample Age (Years)


Regulation of Transcription, 13%
Ion Transport, 3%
Cell Cycle, 8%
Cell Proliferation Regulation, 3%
Apoptosis, 4%
Phosphorylation, 5%
Defense Response, 6%
DNA Repair, 6%

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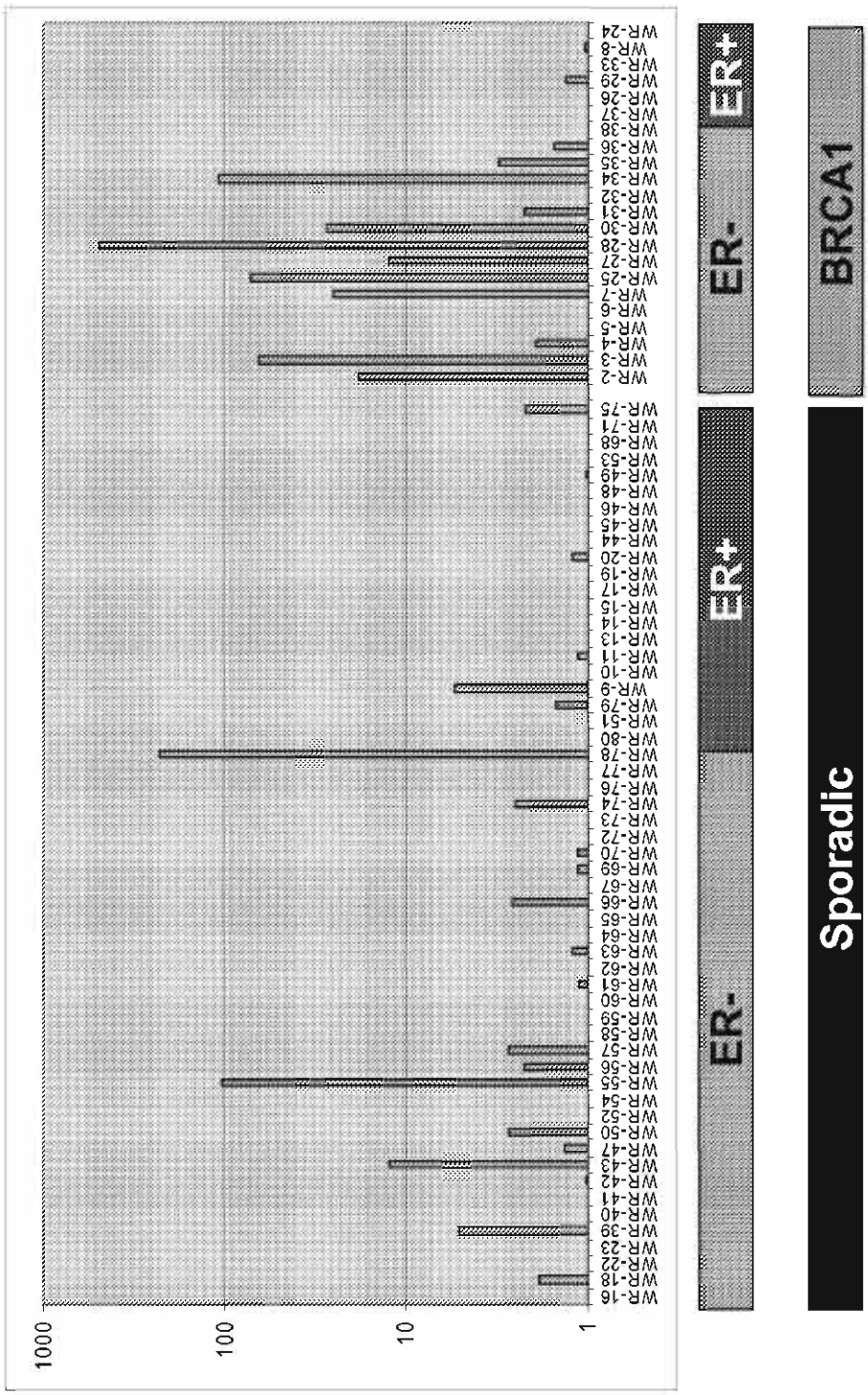

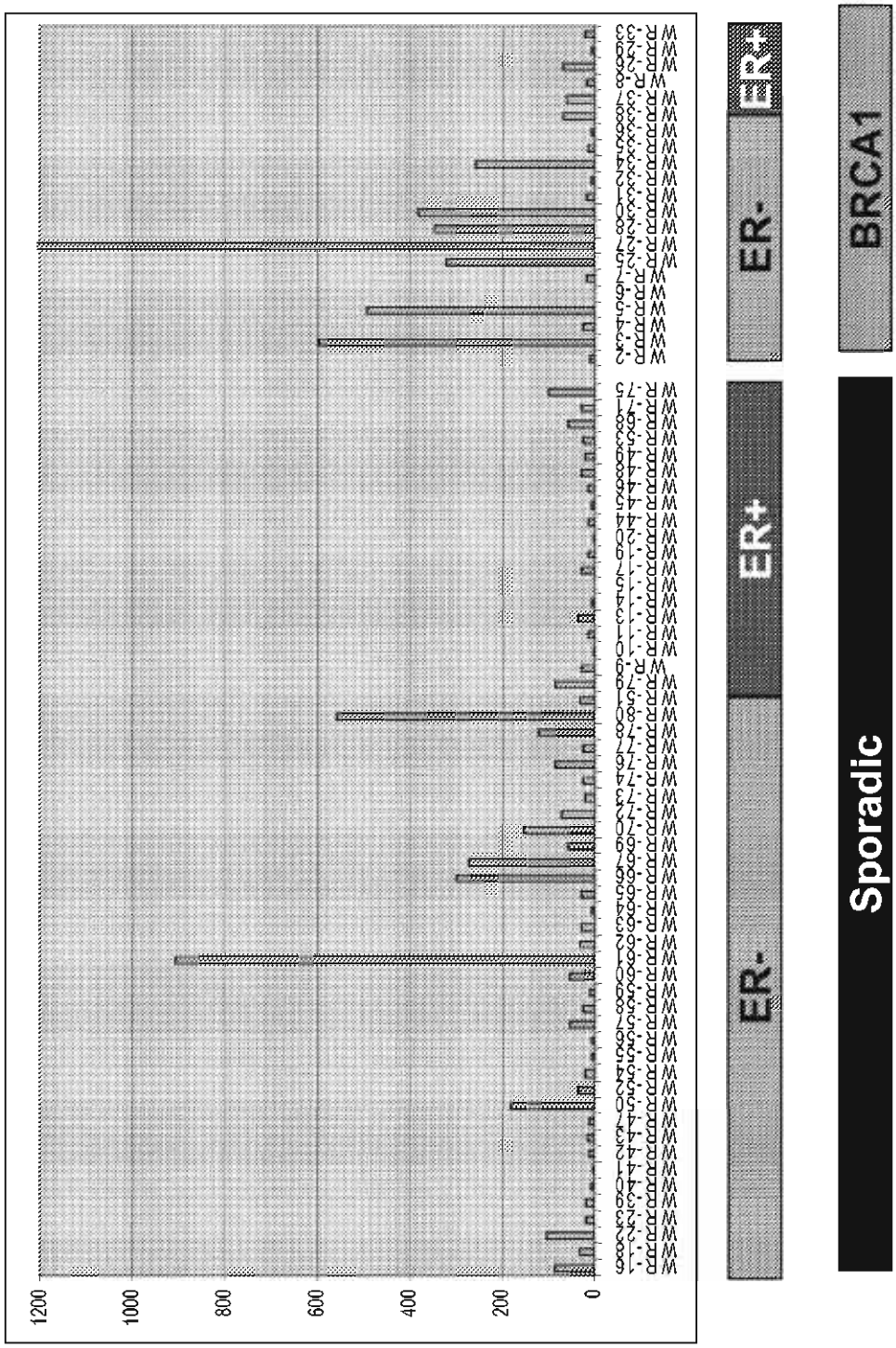
1200
1000
800
600
400
200
0
ER-
ER+
ER-
ER+
Sporadic
BRCA1

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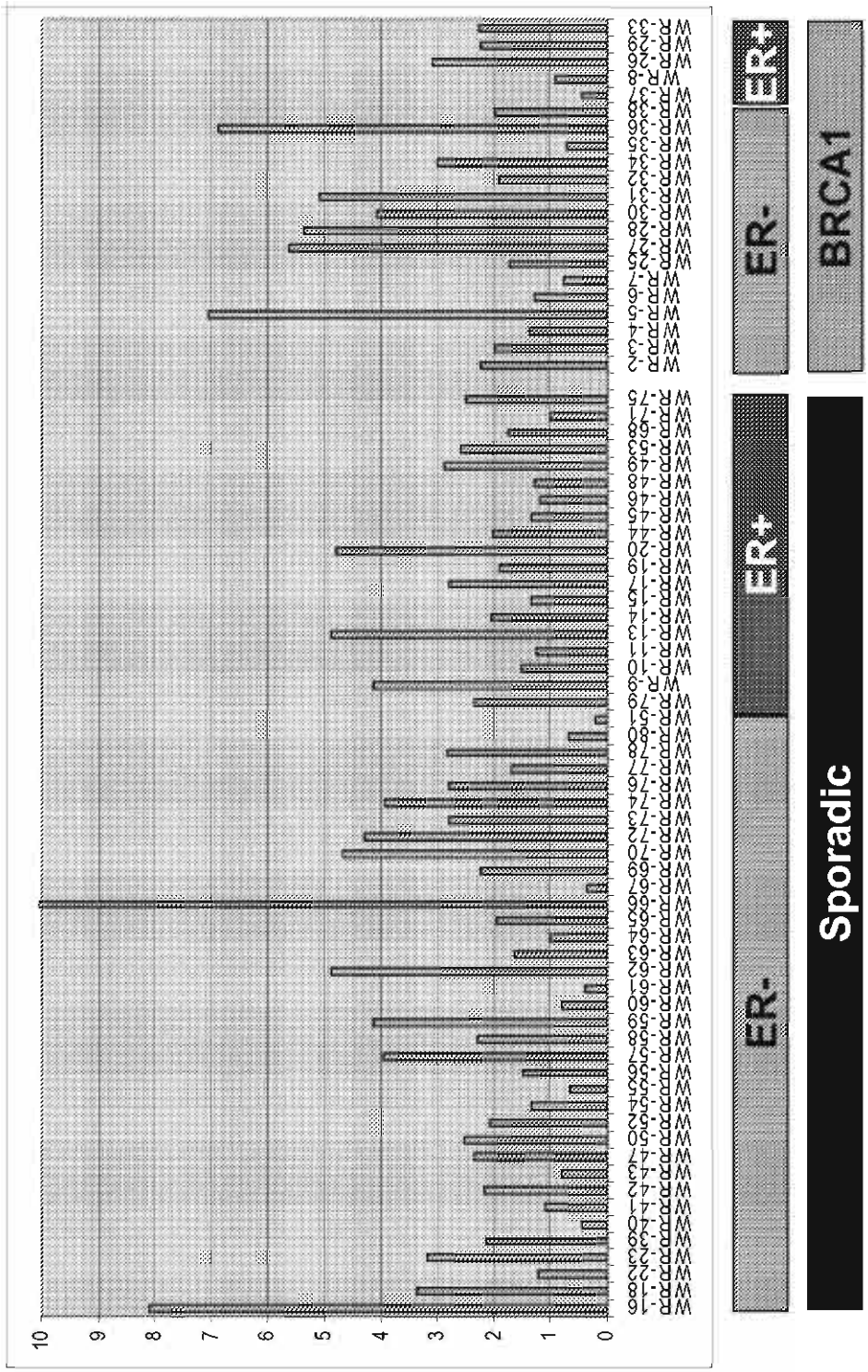

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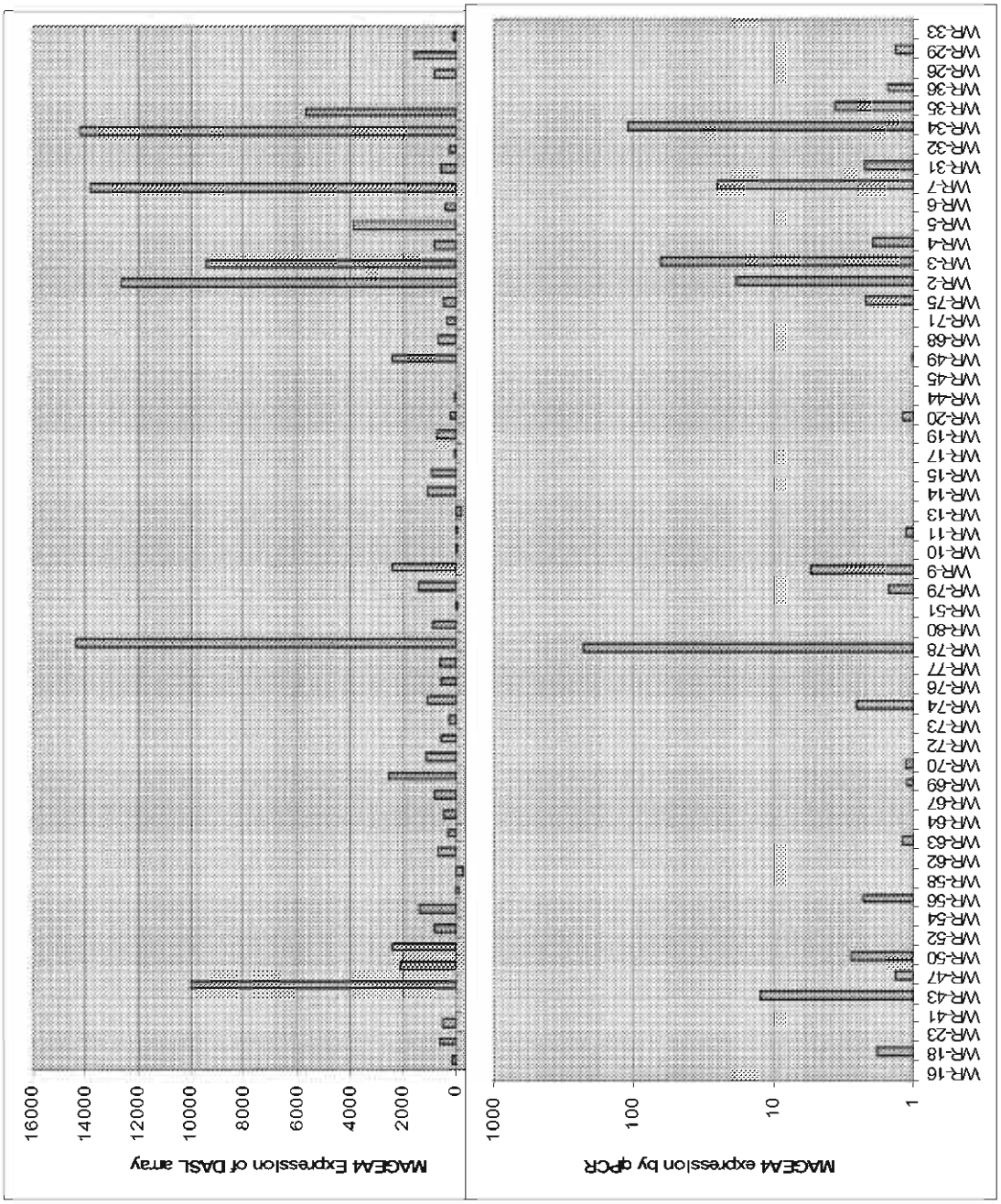
MAGEA4 Expression of DASL array
16000
14000
12000
10000
8000
6000
4000
2000
0
MAGEA4 expression by qPCR
1000
100
10
1
VR-16
VR-18
VR-23
VR-41
VR-43
VR-47
VR-50
VR-52
VR-54
VR-56
VR-58
VR-62
VR-63
VR-64
VR-67
VR-69
VR-70
VR-72
VR-73
VR-74
VR-76
VR-77
VR-78
VR-80
VR-51
VR-79
VR-9
VR-10
VR-11
VR-13
VR-14
VR-15
VR-17
VR-19
VR-20
VR-44
VR-45
VR-49
VR-68
VR-71
VR-75
VR-2
VR-3
VR-4
VR-5
VR-6
VR-7
VR-31
VR-32
VR-34
VR-35
VR-36
VR-26
VR-29
VR-33

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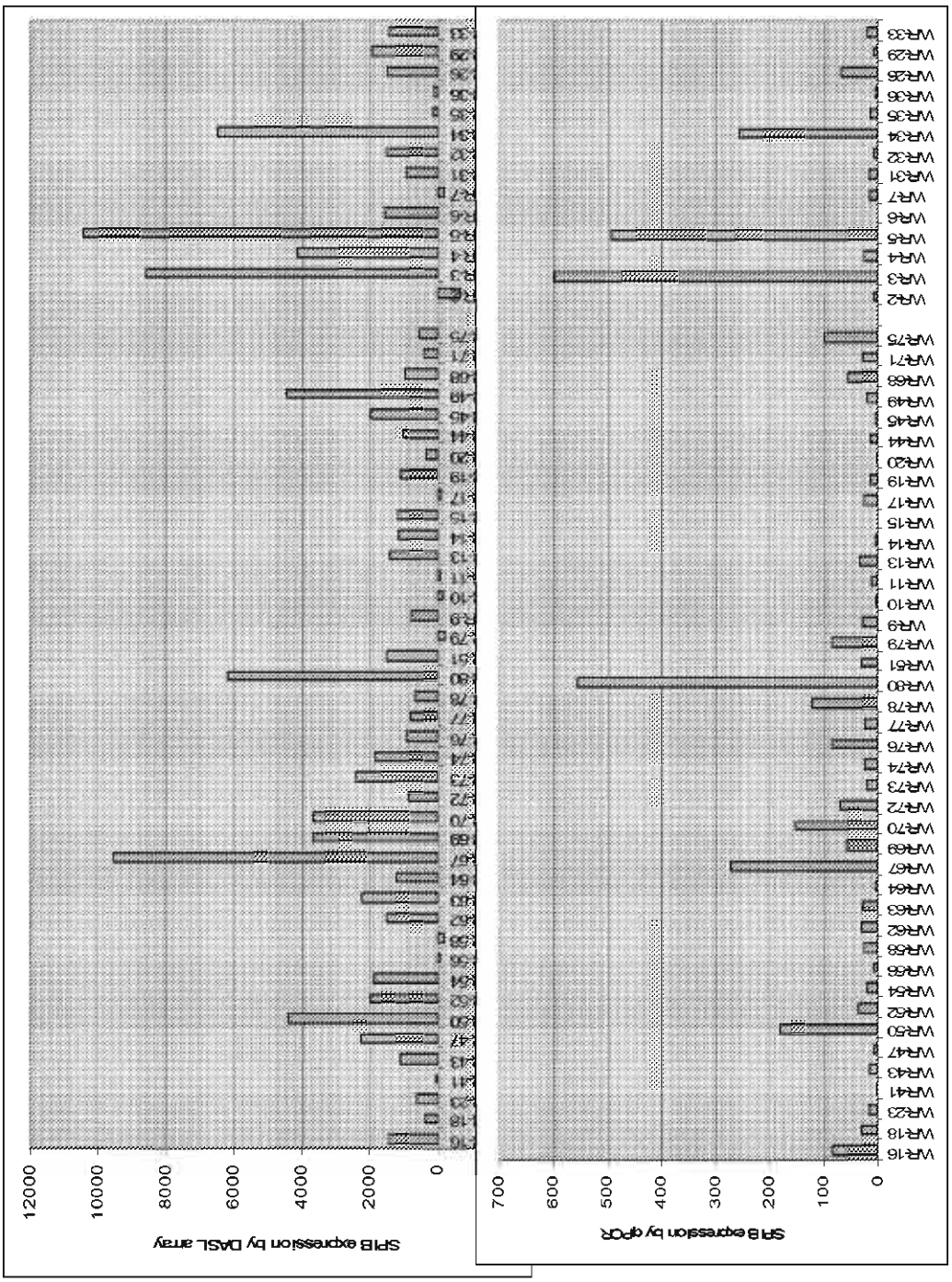
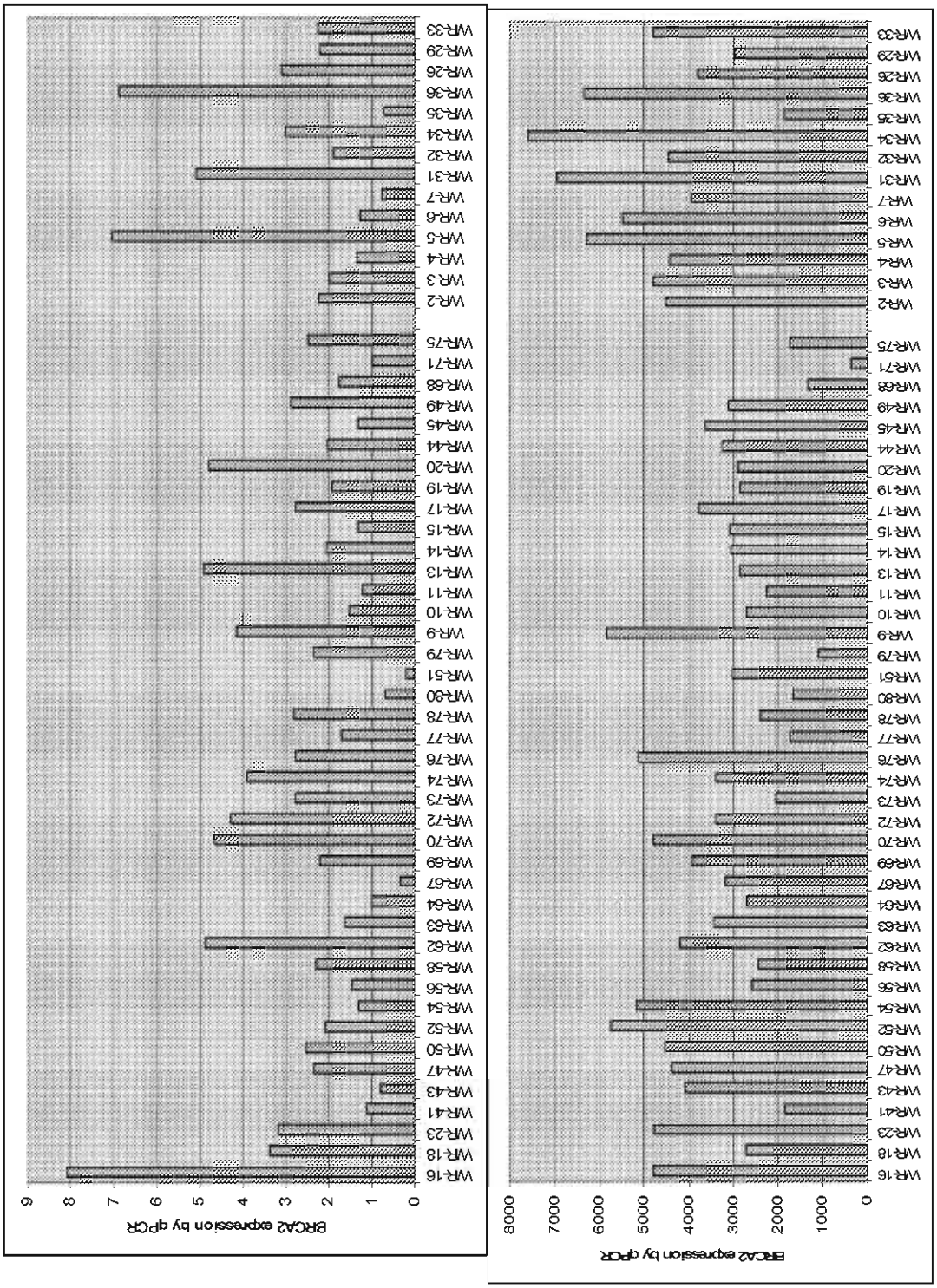

SPIB expression by DASL array
12000
10000
8000
6000
4000
2000
0
SPIB expression by qPCR
700
600
500
400
300
200
100
0


BRCA2 expression by qPCR
0
1000
2000
3000
4000
5000
6000
7000
8000
WR-16
WR-18
WR-23
WR41
WR43
WR47
WR50
WR52
WR54
WR56
WR58
WR62
WR63
WR64
WR67
WR69
WR70
WR72
WR73
WR74
WR76
WR77
WR78
WR80
WR51
WR79
WR9
WR10
WR11
WR13
WR14
WR15
WR17
WR19
WR20
WR44
WR45
WR49
WR68
WR71
WR75
WR2
WR3
WR4
WR5
WR6
WR7
WR31
WR32
WR34
WR35
WR36
WR26
WR29
WR33
BRCA2 expression by qPCR
0
1
2
3
4
5
6
7
8
9
WR-16
WR-18
WR-23
WR41
WR43
WR47
WR50
WR52
WR54
WR56
WR58
WR62
WR63
WR64
WR67
WR69
WR70
WR72
WR73
WR74
WR76
WR77
WR78
WR80
WR51
WR79
WR9
WR10
WR11
WR13
WR14
WR15
WR17
WR19
WR20
WR44
WR45
WR49
WR68
WR71
WR75
WR2
WR3
WR4
WR5
WR6
WR7
WR31
WR32
WR34
WR35
WR36
WR26
WR29
WR33